SAM QUEST- Journal of Emerging Innovations
Vol.1, Issue 1, pp. 54-58, June 2024
Available online at: www.samglobaluniversity.ac.in

Research

Forensic Screening of Medicinal Plants for Qualitative Phytochemical Analysis Using Various Solvent Extracts

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Received: 12/Jun/ 2024; **Accepted**: 17/Jun/2024; **Published**: 25/Jun/2024.

Abstract: Medicinal plants in forensic studies play a very significant role and are rich in bioactive components that are utilized to treat and harm various human ailments. They are crucial to healing as well as to creating artificial injuries. Phytochemical screening is an important step in identifying bioactive compounds present in particular medicinal plants. Hence, in this present work, phytochemical screening of leaf extract of some traditional medicinal plants, such as *Cannabis sativa*, *Ricinus communis*, and *Bryophyllum pinnatum* was carried out. The solvent extracts of the leaves of respective plants were prepared using the Soxhlet apparatus with acetone, chloroform, petroleum ether, and aqueous solvents. Qualitative phytochemical analysis of plants included tests for reducing sugars, flavonoids, steroids, glycosides, polyphenols, tannins, terpenoids, and coumarins. All eight tested phytoconstituents were found present in all three plants in any solvent extracts. Aqueous extract confirmed the presence of a maximum number of phytoconstituents in *C. sativa* in comparison to other solvents. Acetone confirmed the maximum and chloroform confirmed the minimum number of phytoconstituents in *R. communis*, while chloroform confirmed the maximum and aqueous extract confirmed the minimum number of phytoconstituents in *B. pinnatum*. These phytochemicals may be a source of innovative plant-based medications because their existence is connected with the therapeutic potential of these plants. Investigation with a preliminary screening of phytochemicals is used in Forensic Science.

Keywords: *Bryophyllum pinnatum, Cannabis sativa, Ricinus communis,* Forensic science, Phytoconstituents

Introduction

Medicinal plants are an excellent source as they provide a wide variety of possible therapeutic compounds that are both diversified and reasonably safe, compared to manufactured pharmaceuticals. According to the World Health Organization (WHO), traditional plant-based medicines constitute the major source of healthcare for more than 80% of the world's population in developing and underprivileged nations. The WHO has made an effort to identify all internationally used medicinal plants and recognized over 20,000 species. The demand for plant-originated raw materials is increasing at a rate of 15% to 25% annually and is expected to increase by over US$5 trillion by the year 2050. The estimation of total trade by medicinal plants is approximately US$ 1 billion annually. India is incredibly rich in plant species that have therapeutic significance. Most people in society utilize these plants as herbal remedies or as pharmaceutical ingredients in contemporary medicine (Savithramma et al. 2011). Researchers have been concentrating more on herbal remedies recently, and various plants are being investigated for potential therapeutic benefits (Kumari et al. 2017). Collaborative work on forensic botany evidences ethnobotanicals, ethnomedical, ethnopharmacological, and phytochemicals is crucial to attaining research progress in the field of medicinal plants (Yadav and Agarwala 2011). Most of the studies have focused on the phytochemical screening of medicinal plants with an extraction efficiency of one or two

solvents included in this study. The focus point of some of the studies was on the single solvents on multiple plants. So the present study was designed to include the preliminary phytochemical analysis of *C. sativa*, *R. communis*, and *B. pinnatum* and also shows the comparative metabolite extraction efficiency of acetone, chloroform, petroleum ether, and aqueous solvent extracts with the particular plant.

Medicinal Uses of Plant Parts

Cannabis sativa is an important herbaceous plant that originated from Central Asia and has been used in traditional medicine since the dawn of time. The plant has been used medicinally for centuries in a variety of civilizations in the treatment of various ailments. For example, for treating asthma, loss of appetite, depression, and sleeplessness. In modern medicine, it has medical usage in the treatment of anorexia related to HIV/AIDS, nausea and vomiting in cancer chemotherapy, spasticity in multiple sclerosis, gastrointestinal disorders, postpartum hemorrhage, difficulties during child labor, and in the management of sexually transmitted diseases (Selvakumar 2022). There is significant evidence that cannabinoids are also effective in the treatment of several other disorders like neuropathic and chronic pain, movement disorders, and spasms (Ogidigo et al. 2022, Rana et al. 2019).

B. pinnatum is a succulent perennial plant native to Madagascar that has been used in medicines for a long time. *B. pinnatum* and other herb extracts in herbal compositions are said to operate as tonics, boosting health and respiration. The plant shows neurosedative activities, muscle relaxant activities, anticonvulsant activities, nephroprotective, urolithic, antibacterial, antiallergic, antileishmanial, anticonvulsant, anti-inflammatory, anti-ulcer, and analgesic activities. Leaves of *B. pinnatum* also have Neuro-restorative potential.

R. communis is a small woody tree found in India, South Africa, Russia, and Brazil. The root, leaf, and seed oils of this plant have been used in Indian medicine to treat hypoglycemia, liver diseases, and inflammation. The plant parts have anticancer, antidiabetic, antitumor, antiasthmatic, antifertility, bone regeneration, cytotoxicity, antioxidant, insecticidal, antimicrobial, antiprotozoal, and anti-ulcer properties. The seed oil also has a laxative effect and induces labor in pregnant females.

Materials and Methods

Collection of Plant Materials

Fresh leaves of plants free from diseases were collected during January 2023. Taxonomic identification of plants was carried out by the Department of Forensic Science, SAM Global University, Raisen, Madhya Pradesh.

Preparation of Extracts

Collected plant leaves were washed thoroughly with running tap water. Leaf materials were cut down into small pieces and air-dried under shade for 26 days. An electric blender was used to grind the dried plant material into a fine powder and kept in small plastic bags with paper labeling. The crude plant extracts were prepared with different solvents like acetone, petroleum ether, chloroform, and aqueous solvent using the Soxhlet extraction method for approximately 24 hours. The crude extracts were collected and kept in the refrigerator at 5°C in sealed bottles for further use.

Qualitative Phytochemical Analysis

The qualitative analysis of phytochemicals was done for different plant extracts with four different solvents, acetone, petroleum ether, chloroform, and aqueous solvent by using the following standard protocols. The experimental method is illustrated in Fig. 1.

Test for Steroid

Two ml of chloroform was added to the crude extract and concentrated H_2SO_4 was also added side by side. The evolution of red color in the lower chloroform layer directs the presence of steroids. Another test was also conducted, where 2 ml of chloroform was mixed with crude extract. After that 2 ml of acetic acid and 2 ml of concentrated H_2SO_4 were added to the mixture. The appearance of a greenish color depicts the occurrence of steroids in the sample.

Test for Terpenoids

The crude extract was mixed in 2 ml of chloroform and the solution was evaporated to dryness. 2 ml of concentrated H_2SO_4 was then added and the solution heated for another 2 minutes. The appearance of a grayish color indicates the Test for cardiac glycosides (Keller-Kiliani's test)

A few drops of 2% $FeCl_3$ solution were added to glacial acetic acid and 2 ml of this solution was mixed with the crude extract. The mixture wasthen transferred to another vessel having 2 mlof concentrated H_2SO_4. The formation of a brown availability of terpenoids in the sample (Zia et al. 2022, Grotenhermen and Müller-Vahl 2016).

Test for Coumarins

Two ml of 10% sodium hydroxide (NaOH) was mixed with 2 ml of crude extract, the appearance ofayellow color depicts the presence of coumarins (Ogidi et al. 2019, Harborne 1973).

Test for Reducing Sugar (Fehling Test)

One ml of each of Fehling A and Fehling B reagents was mixed and the mixture was then added to the crude extract and the solution boiled. The formation of brick red color precipitates in the bottom of the vessel shows the availability ofreducing sugars in the sample (Zia et al. 2022).

Test for Polyphenols and Tannins

One ml of 2% $FeCl_3$ solution was added to the crude extract. The appearance of a bluish-green or bluish-black color shows the occurrence of polyphenols and tannins in the sample.

Test for Flavonoids

The crude extract was mixed with a few small pieces of magnesium ribbon and then concentrated Hydrochloric acid was added to it drop by drop. After a few minutes, the appearance of pink or magenta color indicates the availability of flavonoids in the sample.

Test for Glycosides (Salkowski's Test)

Two ml of chloroform was added to the crude extract. Then 2 ml of concentrated H_2SO_4 was addedand gently shaken. A reddish brown color shows theoccurrence of the steroidal ring, i.e., the glycone portion ofthe glycoside. The color ring at the interface indicates the availability of cardiac glycosides in the sample.

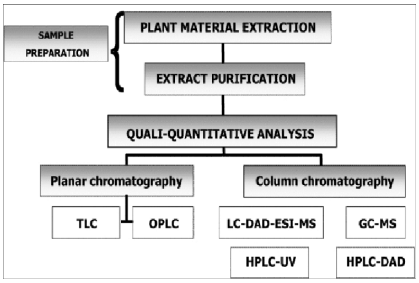

Fig. 1. A schematic diagram showing the experimental method.

Results and Discussion

The phytochemical characteristics of the leaf extract of *C. sativa* tested with different solvent extracts are summarized in Table 1, which shows the presence of medically active compounds in the plant. Reducing sugars, glycosides, cardiac glycosides, polyphenols, tannins, flavonoids, steroids, terpenoids, and coumarins, all were found present in different solvent extracts. Aqueous extract yielded more metabolites in comparison to acetone, chloroform, and petroleum ether extracts of *C. sativa*. Comparative studies for the same plant are shown in Table 2, which demonstrate the presence of most of the phytoconstituents confirmed by our study, but the solvents were not similar. Studies confirmed the maximum yield with aqueous extract.

Table 1. Phytochemical analysis of leaf extracts of *C. sativa.*

Phyto-constituents	Acetone	Chloroform	Petroleum ether	Aqueous
Reducing sugars	-	-	+	-
Glycosides	-	-	+	+
Cardiac glycosides	-	-	-	+
Polyphenols and Tannins	+	+	+	+
Flavonoids	+	-	-	-
Steroids	+	-	+	+
Terpenoids	-	+	-	-
Coumarins	+	+	-	+

Present (+), Absent (-)

Table 2. Comparative studies (*C. sativa*). Source: Nandagoapalan et al. 2016, Kulkarni and Mane 2019, Mekuria et al. 2017.

Plant part	Solvent	Phyto-constituents
Leaf, Stem, Root	Chloroform, Alcohol Aqueous, n-hexane	steroids, resins, fixed oil, alkaloids, flavonoids, terpenoids, tannin, amino acids, proteins, glycosides, phenol, saponins
Leaf	-	Alkaloids, flavonoids, cardiac glycosides, resins, terpenes, steroids
Leaf	-	phenol, saponins, Alkaloids, flavonoids, glycosides, steroids
Leaf	-	Anthocyanines, Steroids, Terpenoids

Table 3. Phytochemical analysis of leaf extracts of *R. communis*

Phytochemical test	Acetone	Chloroform	Petroleum ether	Aqueous
Reducing sugars	+	+	+	-
Glycosides	-	+		
Cardiac glycosides	+	-	+	+
Polyphenols and Tannins	+	+	+	+
Flavonoid	+	-	+	+
Steroids	+	+	+	+
Terpenoids	+	-	+	+
Coumarins	+	+	+	-

The phytochemical characteristics of *R. communis* tested with different solvent extracts are summarized in Table 3. Medically active compounds, reducing sugars, glycosides, cardiac glycosides, Polyphenols, tannins, flavonoids, steroids, terpenoids, and Coumarins, all were found present in different solvent extracts. For *R. communis*, all the phyto-constituents were found present in acetone extract, glycosides(1 compound) were found absent in petroleum ether extract, reducing sugars and Coumarins (2 compounds) were found absent in aqueous extract, while cardiac glycosides, flavonoids and terpenoids (3 compounds) were found absent in chloroform extract. Results revealed that acetone extract yielded maximum and chloroform extract yielded minimum numbers of constituents. Various studies on the phytochemical analysis of *R. communis* are summarized in Table 4, which shows the presence of similar compounds in different parts of the plant with the same or different solvent extracts. Most of the studies don't reveal the extraction efficiency of solvents with particular metabolites, which can be treated as a drawback of that study.

Table 4. Comparative studies (*R. communis*).

Plant Part	Solvent	Phyto-constituents
Leaf	Water, Methanol, Ethanol, Acetone	Proteins Carbohydrates Phenol/Tannins, Alkaloids, Flavonoid, Steriods,
Leaf	-	Flavonoid Saponins Glycosides Steroids, Phenol, Tannins, Saponins, Starch
Seed, Root, Leaf	-	Phenol, Flavonoid, Glycosides, Steroid
Seed Oil	-	Alkaloids, Terpenoids, Cardiac Glycosides, Tannins, Steroids, Saponins

The results of phytochemical analysis of different solvent extracts of *B. pinnatum* are shown in Table 5. Results revealed the presence of all tested compounds in the plant, chloroform extract shows the presence of all Phytoconstituents, while reducing sugars were absent in acetone extract and flavonoids were found absent in petroleum ether extract. Four compounds were absent in the aqueous extract. It implies chloroform extract yielded maximum and aqueous extract yielded minimum metabolites in *B. pinnatum*. Table 6 is compiled on the basis of some previous studies, which show similar results for the same and different solvent extracts for the leaf and other parts of the plant. In the case of different plants, the phytoconstituents extraction efficiency of differentsolvents varies greatly.

Table 5. Phytochemical analysis of leaf extracts of *B. pinnatum.*

Plant Part	Solvent	Phyto-constituents
Leaf	Water, methanol, ethanol, acetone	Proteins, Carbohydrates, Phenols/Tannins, Flavonoids, Saponins, Glycosides, Steroids, Alkaloids,
Wood, Stem bark	Hexane, ethyl acetate, methanol	Reducing sugars, saponins, steroids, tannins, alkaloids, flavonoids, phenols
Leaf, Root, Stem	-	Alkaloid, Tannin, Saponin, Flavonoid, Terpenoid, Glycoside, Phenols
Leaf	-	Flavonoid, Glycoside, Alkaloids, Triterpenoids, Tannins, Phenolic

Table 6, Comparative studies (*B. pinnatum*).

Phytochemical Test	Acetone	Chloroform	Petroleum Ether	Aqueous
Reducing sugars	-	+	+	+
Glycosides	+	+	+	-
Cardiac glycosides	+	+	+	-
Polyphenols and Tannins	+	+	+	+
Flavonoid	+	+	-	-
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Coumarins	+	+	+	+

Conclusion

The majority of the biologically active phytochemicals were found present in acetone, petroleum ether, chloroform, and aqueous extracts of leaves of *C. sativa*, *R. communis*, and *B. pinnatum. R. communis*, and *B. pinnatum* were more phytochemically rich in comparison to *C. sativa*. The medicinal plants were found rich in secondary metabolites, commonly employed in conventional medicine to treat

and combat a wide range of illnesses. The antispasmodic, anti-inflammatory, analgesic, diuretic, and many other properties can be imputed to their high availability of polyphenols, flavonoids, tannins, terpenoids, steroids, glycosides, coumarins, and reducing sugars. The research carried out by us confirmed the therapeutic qualities of these plant species. It will be useful to do more research in the field of the quantitative analysis of these phyto-compounds. Our study can be used as scientific support for the formulation of a variety of medications.

Acknowledgments

The authors acknowledge the support of SAM Global University, Raisen during the study.

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